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(54) Title: TREATMENT OF CONDITIONS WITH A NEED FOR THE INHIBITION OF GSK-3 (57) Abstract <p>A method of treatment and/or prophylaxis of conditions associated with a need for the inhibition of glycogen synthase kinase-3 (GSK-3), which method comprises the administration of certain maleimide or carbazole compounds, or pharmaceutically acceptable derivatives thereof, and the use of such compounds in the manufacture of a medicament for the treatment of conditions associated with the need for GSK-3 inhibition.</p>		

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TREATMENT OF CONDITIONS WITH A NEED FOR THE INHIBITION OF GSK-3

This invention relates to a novel method for the treatment and/or prophylaxis of conditions associated with a need for the inhibition of glycogen synthase kinase-3 (GSK-3).

GSK-3 is a serine/threonine protein kinase composed of two isoforms (α and β) which are encoded by distinct genes. GSK-3 is one of several protein kinases which phosphorylates glycogen synthase (GS) (Embi *et al* Eur. J. Biochem. (107) 519-527 (1980)). The α and β isoforms have a monomeric structure of 49 and 47kD respectively and are both found in mammalian cells. Both isoforms phosphorylate muscle glycogen synthase (Cross *et al* Biochemical Journal (303) 21-26 (1994)) and these two isoforms show good homology between species (e.g. human and rabbit GSK-3 α are 96% identical).

Type II diabetes (or Non-Insulin Dependent Diabetes Mellitus, NIDDM) is a multifactorial disease. Hyperglycaemia is due to insulin resistance in the liver, muscle and other tissues coupled with inadequate or defective secretion of insulin from pancreatic islets. Skeletal muscle is the major site for insulin-stimulated glucose uptake and in this tissue, glucose removed from the circulation is either metabolised through glycolysis and the TCA cycle, or stored as glycogen. Muscle glycogen deposition plays the more important role in glucose homeostasis and Type II diabetic subjects have defective muscle glycogen storage.

The stimulation of glycogen synthesis by insulin in skeletal muscle results from the dephosphorylation and activation of glycogen synthase (Villar-Palasi C. and Larner J. Biochim. Biophys. Acta (39) 171-173 (1960), Parker P J *et al* Eur. J. Biochem. (130) 227-234 (1983), and Cohen P. Biochem. Soc. Trans. (21) 555-567 (1993)). The phosphorylation and dephosphorylation of GS are mediated by specific kinases and phosphatases. GSK-3 is responsible for phosphorylation and deactivation of GS, while glycogen bound protein phosphatase 1 (PP1G) dephosphorylates and activates GS. Insulin both inactivates GSK-3 and activates PP1G (Srivastava A K and Pandey S K Mol. and Cellular Biochem. (182) 135-141 (1998)).

Chen *et al* Diabetes (43) 1234-1241 (1994) found that there was no difference in the mRNA abundance of PP1G between patients with Type II diabetes and control patients, suggesting that an increase in GSK-3 activity might be important in Type II diabetes. It has also recently been demonstrated that GSK-3 is overexpressed in Type II diabetic muscle and that an inverse correlation exists between skeletal muscle GSK-3 α activity and insulin action (Nikoulina *et al* Glycogen Synthase Kinase-3 in Human Skeletal Muscle: Relationship To Insulin Resistance in Type II Diabetes. Diabetes (47(1)) 0028 Page A7 (1998)

(Oral presentation)). Overexpression of GSK-3 β and constitutively active GSK-3 β (S9A, S9E) mutants in HEK-293 cells resulted in suppression of glycogen synthase activity (Eldar-Finkelman *et al* PNAS (93) 10228-10233 (1996)) and overexpression of GSK-3 β in CHO cells, expressing both insulin receptor and insulin receptor substrate 1 (IRS-1), resulted in an impairment of insulin action (Eldar-Finkelman and Krebs PNAS (94) 9660-9664 (1997)). Recent evidence for the involvement of elevated GSK-3 activity and the development of insulin resistance and type II diabetes in adipose tissue has emerged from studies undertaken in diabetes and obesity prone C57BL/6J mice (Eldar-Finkelman *et al* Diabetes (48) 1662-1666 (1999)).

GSK-3 has been shown to phosphorylate other proteins *in vitro* including the eukaryotic initiation factor eIF-2B at Serine⁵⁴⁰ (Welsh *et al* FEBS Letts (421) 125-130 (1998)). This phosphorylation results in an inhibition of eIF-2B activity and leads to a reduction in this key regulatory step of translation. In disease states, such as diabetes, where there is elevated GSK-3 activity this could result in a reduction of translation and potentially contribute to the pathology of the disease.

Several aspects of GSK-3 functions and regulation in addition to modulation of glycogen synthase activity indicate that inhibitors of this enzyme may be effective in treatment of disorders of the central nervous system. GSK-3 activity is subject to inhibitory phosphorylation by PI 3 kinase-mediated or Wnt-1 class-mediated signals that can be mimicked by treatment with lithium, a low mM inhibitor of GSK-3 (Stambolic V., Ruel L., and Woodgett J.R. Curr. Biol. 1996 6(12): 1664-8).

GSK-3 inhibitors may be of value as neuroprotectants in treatment of acute stroke and other neurotraumatic injuries. Roles for PI 3-kinase signalling through PKB/akt to promote neuronal cell survival are well established, and GSK-3 is one of a number of PKB/akt substrates to be identified that can contribute to the inhibition of apoptosis via this pathway (Pap and Cooper, (1998) J. Biol. Chem. 273: 19929-19932). Evidence suggests that astrocytic glycogen can provide an alternative energy source to facilitate neuronal survival under conditions of glucose deprivation (for example see Ransom B.R. and Fern R. (1997) Glia 21: 134-141 and references therein). Lithium is known to protect cerebellar granule neurons from death (D' Mello *et al* (1994) Exp. Cell Res. 211: 332-338 and Volonte *et al* (1994) Neurosci. Letts. 172: 6-10) and chronic lithium treatment has demonstrable efficacy in the middle cerebral artery occlusion model of stroke in rodents (Nonaka and Chuang (1998) Neuroreport 9(9): 2081-2084). Wnt-induced axonal spreading and branching in neuronal culture models has been shown to correlate with GSK-3 inhibition (Lucas and Salinas (1997) Dev. Biol.

192: 31-44) suggesting additional value of GSK-3 inhibitors in promoting neuronal regeneration following neurotraumatic insult.

Tau and β -catenin, two known *in vivo* substrates of GSK-3, are of direct relevance in consideration of further aspects of the value of GSK-3 inhibitors in relation to treatment of chronic neurodegenerative conditions. Tau hyperphosphorylation is an early event in neurodegenerative conditions such as Alzheimer's disease (AD), and is postulated to promote microtubule disassembly. Lithium has been reported to reduce the phosphorylation of tau, enhance the binding of tau to microtubules, and promote microtubule assembly through direct and reversible inhibition of glycogen synthase kinase-3 (Hong M., Chen D.C., Klein P.S. and Lee V.M. J.Biol. Chem. 1997 272(40) 25326-32). β -catenin is phosphorylated by GSK-3 as part of a tripartite complex with axin, resulting in β -catenin being targeted for degradation (Ikeda *et al* (1998) EMBO J. 17: 1371-1384). Inhibition of GSK-3 activity is a key mechanism by which cytosolic levels of catenin are stabilised and hence promote β -catenin-LEF-1/TCF transcriptional activity (Eastman and Grosschedl (1999) Curr. Opin. Cell Biol. 11: 233). Rapid onset AD mutations in presenilin-1 (PS-1) have been shown to decrease the cytosolic β -catenin pool in transgenic mice. Further evidence suggests that such a reduction in available β -catenin may increase neuronal sensitivity to amyloid mediated death through inhibition of β -catenin-LEF-1/TCF transcriptional regulation of neuroprotective genes (Zhang *et al* (1998) Nature **395**: 698-702). A likely mechanism is suggested by the finding that mutant PS-1 protein confers decreased inactivation of GSK-3 compared with normal PS-1 (Weihl C.C., Ghadge G.D., Kennedy S.G., Hay N., Miller R.J., and Roos R.P.(1999) J. Neurosci. **19**: 5360-5369).

WO 97/41854 (University of Pennsylvania) discloses that an effective drug for the treatment of manic depression is lithium, but that there are serious drawbacks associated with this treatment. Whilst the precise mechanism of action of this drug for treatment of manic depression remains to be fully defined, current models suggest that inhibition of GSK-3 is a relevant target that contributes to the modulation of AP-1 DNA binding activity observed with this compound (see Manji *et al* (1999) J. Clin. Psychiatry **60** (suppl 2): 27-39 for review).

GSK-3 inhibitors may also be of value in treatment of schizophrenia. Reduced levels of β -catenin have been reported in schizophrenic patients (Cotter D, Kerwin R, al-Sarraj S, Brion JP, Chadwich A, Lovestone S, Anderton B, and Overall I. 1998 Neuroreport **9**:1379-1383) and defects in pre-pulse inhibition to startle response have been observed in schizophrenic patients (Swerdlow *et al* (1994) Arch. Gen. Psychiat. **51**: 139-154). Mice lacking the adaptor protein dishevelled-1, an essential mediator of Wnt-induced inhibition of GSK-3, exhibit

both a behavioural disorder and defects in pre-pulse inhibition to startle response (Lijam N, Paylor R, McDonald MP, Crawley JN, Deng CX, Herrup K, Stevens KE, Maccaferri G, McBain CJ, Sussman DJ, and Wynshaw-Boris A. (1997) *Cell* **90**: 895-905). Together, these findings implicate deregulation of GSK-3 activity as contributing to schizophrenia. Hence, small molecule inhibitors of GSK-3 catalytic activity may be effective in treatment of this mood disorder.

The finding that transient β -catenin stabilisation may play a role in hair development (Gat *et al* *Cell* (**95**) 605-614(1998)) suggests that GSK-3 inhibitors could be used in the treatment of baldness.

Published Patents and Patent Applications, EP 470490 (Roche), WO 93/18766 (Wellcome), WO 93/18765 (Wellcome), EP 397060 (Goedecke), WO 98/11105 (Astra), WO 98/11103 (Astra), WO 98/11102 (Astra), WO 98/04552 (Roche), WO 98/04551 (Roche), DE 4243321 (Goedecke), DE 4005970 (Boehringer), DE 3914764 (Goedecke), WO 96/04906 (Wellcome), WO 95/07910 (Wellcome), DE 4217964 (Goedecke), US 5856517 (Roche), US 5891901 (Roche), and WO 99/42100 (Sagami) (which Patents and Patent Applications are hereinafter also referred to as the "Publications of Group (IA)") disclose certain bisindole maleimides, indole aryl maleimides, and indolocarbazoles (hereinafter also referred to as the "Compounds of Group (IA)") and methods for their preparation.

Published Patents and Patent Applications EP 328026 (Roche), EP 384349 (Roche), EP 540956 (Roche), and DE 4005969 (Boehringer) (which Patents and Patent Applications are hereinafter also referred to as the "Publications of Group (IB)") disclose certain bisindole maleimides, indole aryl maleimides, and indolocarbazoles (hereinafter also referred to as the "Compounds of Group (IB)") and methods for their preparation.

Published Patent Application EP 508792 (Schering) (which Patent Application is hereinafter also referred to as the "Publication of Group (IC)") discloses certain maleimide derivatives (hereinafter also referred to as the "Compounds of Group (IC)") and methods for their preparation.

The group of publications consisting of the "Publications of Group (IA)", the "Publications of Group (IB)", and the "Publications of Group (IC)" is hereinafter referred to as the "Publications of Group (I)".

The group of compounds consisting of the "Compounds of Group (IA)", the "Compounds of Group (IB)", and the "Compounds of Group (IC)" is hereinafter referred to as the "Compounds of Group (I)".

Published Patents and Patent Applications WO 95/17182 (Lilly), WO 95/35294 (Lilly), EP 624586 (Roche), EP 657458 (Lilly), EP 776899 (Lilly), EP 805158 (Lilly), US 5491242 (Lilly), US 5541347 (Lilly), US 5545636 (Lilly), US

5552396 (Lilly), US 5624949 (Lilly), US 5710145 (Lilly), US 5721272 (Lilly), WO 97/18809 (Lilly), and WO 98/07693 (Lilly) (which Patents and Patent Applications are hereinafter also referred to as the "Publications of Group (II)") disclose certain compounds (hereinafter also referred to as the "Compounds of Group (II)") which are selective Protein Kinase C (PKC) beta 1 and PKC beta 2 inhibitors which are stated to be useful in the treatment of conditions associated with diabetes mellitus and complications thereof.

Hers *et al* FEBS Letters 460 (1999) 433-436 disclose certain bisindolylmaleimides as inhibitors of GSK-3.

The disclosures of the "Publications of Group (I)" and the "Publications of Group (II)" are incorporated herein by reference.

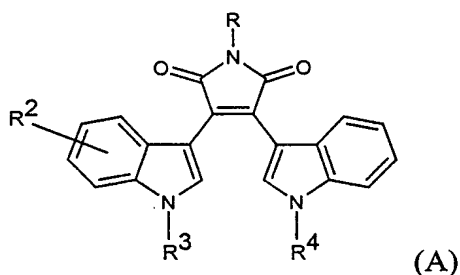
It has now been discovered that a series of certain bisindole maleimides, indole aryl maleimides, and indolocarbazoles are particularly potent and selective inhibitors of GSK-3. These compounds are indicated to be useful for the treatment and/or prophylaxis of conditions associated with a need for the inhibition of GSK-3, such as diabetes and conditions associated with diabetes, chronic neurodegenerative conditions including dementias such as Alzheimer's disease, manic depression, mood disorders such as schizophrenia, neurotraumatic diseases such as acute stroke, hair loss, and cancer.

Accordingly, in a first aspect, the present invention provides a method for the treatment and/or prophylaxis of conditions associated with a need for the inhibition of GSK-3, such as diabetes and conditions associated with diabetes, chronic neurodegenerative conditions including dementias such as Alzheimer's disease, manic depression, mood disorders such as schizophrenia, neurotraumatic diseases such as acute stroke, hair loss, and cancer, which method comprises the administration of a pharmaceutically effective, non-toxic amount of a compound selected from the "Compounds of Group (I)".

A suitable compound selected from the "Compounds of Group (I)" is a compound of formula (I) as respectively defined in EP 470490, WO 93/18766, WO 93/18765, EP 397060, WO 98/11105, WO 98/11103, WO 98/11102, WO 98/04552, WO 98/04551, DE 4243321, DE 4005970, DE 3914764, WO 96/04906, WO 95/07910, DE 4217964, US 5856517, US 5891901, WO 99/42100, EP 328026, EP 384349, EP 540956, DE 4005969, or EP 508792 (the "Publications of Group (I)").

In particular, a compound selected from the "Compounds of Group (I)" includes a compound selected from those compounds specifically disclosed as examples in the "Publications of Group (I)".

An example of a compound selected from the "Compounds of Group (I)" is a compound selected from those disclosed in the "Publications of Group (IA)" or the "Publications of Group (IB)", and is of formula (A):



wherein

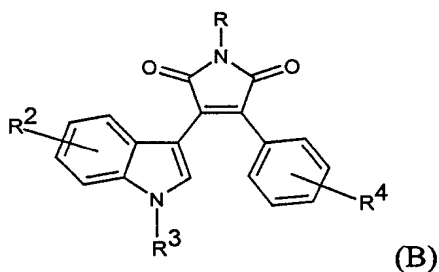
R is hydrogen;

R² is hydrogen, 5-OPrⁿ, 5-Ph, 5-CO₂Me or 5-NO₂;

R³ is Me or (CH₂)₃OH, and;

R⁴ is Me, Prⁿ, -(CH₂)₃X wherein X is selected from CN, NH₂, CO₂H, CONH₂, or OH.

A further example of a compound selected from the "Compounds of Group (I)" is a compound selected from those disclosed in the "Publications of Group (IB)" and is of formula (B):



wherein

R is hydrogen;

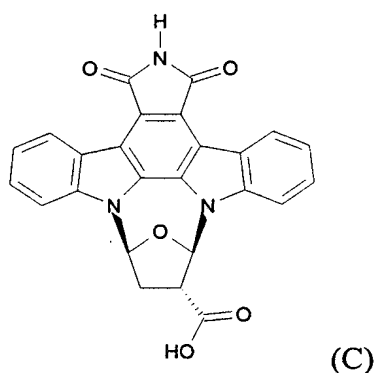
R² is hydrogen;

R³ is Me or a group -(CH₂)₃Y wherein Y is NH₂ or OH, and;

R⁴ is 2-Cl or 2,4-di-Cl.

Yet a further example of a compound selected from the "Compounds of Group (I)" is a compound selected from those disclosed in the "Publications of Group (IC)" and is 9,10,11,12-tetrahydro-10-carboxy-9,12,-epoxy-1H-

diindolo[1,2,3-fg:3',2',1'-kl]pyrrolo[3,4-i]benzodiazocine-1,3(2H)-dione (formula (C)).



In a further aspect, the present invention provides a method for the treatment and/or prophylaxis of conditions associated with a need for the inhibition of GSK-3, such as diabetes and conditions associated with diabetes, chronic neurodegenerative conditions including dementias such as Alzheimer's disease, manic depression, mood disorders such as schizophrenia, neurotraumatic diseases such as acute stroke, hair loss, and cancer which method comprises the administration of a pharmaceutically effective, non-toxic amount of a compound selected from the "Compounds of Group (II)", but providing that the method does not encompass the treatment of conditions associated with diabetes mellitus or complications thereof.

A suitable compound selected from the "Compounds of Group (II)" is a compound of formula (I) as defined in WO 95/17182, WO 95/35294, EP 624586, EP 657458, EP 776899, EP 805158, US 5491242, US 5541347, US 5545636, US 5552396, US 5624949, US 5710145, US 5721272, WO 97/18809, or WO 98/07693 (the "Publications of Group (II)")

In particular, a compound selected from the "Compounds of Group (II)" includes a compound selected from those compounds specifically disclosed as examples in the "Publications of Group (II)".

Examples of compounds of formula (A) include those on the list below (hereinafter referred to as "List A"):

- 3,4-bis(1-methyl-3-indolyl)pyrrole-2,5-dione;
- 3-(1-methyl-3-indolyl)-4-(1-propyl-3-indolyl)pyrrole-2,5-dione;
- 3-(1-methyl-3-indolyl)-4-(1-[3-cyanopropyl]-3-indolyl)pyrrole-2,5-dione;
- 3-(1-methyl-3-indolyl)-4-(1-[3-aminopropyl]-3-indolyl)pyrrole-2,5-dione;
- 3-(1-methyl-3-indolyl)-4-(1-[3-carboxypropyl]-3-indolyl)pyrrole-2,5-dione;

3-(1-methyl-3-indolyl)-4-(1-[3-carbamoylpropyl]-3-indolyl)pyrrole-2,5-dione;
 3-(1-methyl-5-propyloxy-3-indolyl)-4-(1-[3-aminopropyl]-3-indolyl)pyrrole-2,5-dione;
 3-(1-methyl-5-phenyl-3-indolyl)-4-(1-[3-hydroxypropyl]-3-indolyl)pyrrole-2,5-dione;
 3-(1-methyl-5-phenyl-3-indolyl)-4-(1-[3-aminopropyl]-3-indolyl)pyrrole-2,5-dione;
 3-(1-methyl-5-methoxycarbonyl-3-indolyl)-4-(1-[3-hydroxypropyl]-3-indolyl)pyrrole-2,5-dione;
 3-(1-methyl-5-nitro-3-indolyl)-4-(1-[3-hydroxypropyl]-3-indolyl)pyrrole-2,5-dione, and;
 3-(1-[3-hydroxypropyl]-5-nitro-3-indolyl)-4-(1-methyl-3-indolyl)pyrrole-2,5-dione;
 or a pharmaceutically acceptable derivative thereof.

Examples of compounds of formula (B) include those on the list below (hereinafter referred to as "List B"):

3-(1-methyl-3-indolyl)-4-(2-chlorophenyl)pyrrole-2,5-dione;
 3-(1-methyl-3-indolyl)-4-(2,4-dichlorophenyl)pyrrole-2,5-dione;
 3-(1-[3-hydroxypropyl]-3-indolyl)-4-(2-chlorophenyl)pyrrole-2,5-dione, and;
 3-(1-[3-aminopropyl]-3-indolyl)-4-(2-chlorophenyl)pyrrole-2,5-dione;
 or a pharmaceutically acceptable derivative thereof.

The example compound of formula (C) is:

10,11,12-tetrahydro-10-carboxy-9,12-epoxy-1H-diindolo[1,2,3-fg:3',2',1'-kl]pyrrolo[3,4-i]benzodiazocine-1,3(2H)-dione, or a pharmaceutically acceptable derivative thereof.

Suitably, a compound selected from the "Compounds of Group (I)" is a compound selected from those disclosed in the "Publications of Group (IA)" or the "Publications of Group (IB)" and is of formula (A) as hereinbefore defined.

Suitably, a compound selected from the "Compounds of Group (I)" is a compound selected from those disclosed in the "Publications of Group (IC)" and is of formula (C) as hereinbefore defined.

Favourably, a compound selected from the "Compounds of Group (I)" is a compound of formula (A) selected from "List A".

Favourably, a compound selected from the "Compounds of Group (I)" is 10,11,12-tetrahydro-10-carboxy-9,12-epoxy-1H-diindolo[1,2,3-fg:3',2',1'-kl]pyrrolo[3,4-i]benzodiazocine-1,3(2H)-dione or a pharmaceutically acceptable derivative thereof.

Preferably, a compound selected from the "Compounds of Group (I)" is a compound selected from those disclosed in the "Publications of Group (IB)" and is of formula (B) as hereinbefore defined.

More preferably, a compound selected from the "Compounds of Group (I)" is a compound of formula (B) selected from "List B".

Most preferably, a compound selected from the "Compounds of Group (I)" is 3-(1-methyl-3-indolyl)-4-(2,4-dichlorophenyl)pyrrole-2,5-dione.

Certain of the "Compounds of Group (I)" and the "Compounds of Group (II)" may contain at least one chiral atom and/or may contain multiple bonds and hence may exist in one or more stereoisomeric forms.

The present invention encompasses all of the isomeric forms of the "Compounds of Group (I)" and the "Compounds of Group (II)" including enantiomers and geometric isomers whether as individual isomers or as mixtures of isomers, including racemic modifications.

The present invention also includes the pharmacologically active derivatives of the "Compounds of Group (I)" and the "Compounds of Group (II)" as described in the "Publications of Group (I)" and the "Publications of Group (II)" respectively.

Suitable pharmacologically active derivatives of the compounds of the invention include salts and solvates as described in the "Publications of Group (I)" and the "Publications of Group (II)".

Suitable pharmaceutically acceptable derivatives of the "Compounds of Group (I)" and the "Compounds of Group (II)" include pharmaceutically acceptable salts and pharmaceutically acceptable solvates.

The present invention further provides a compound selected from the "Compounds of Groups (I)" or the "Compounds of Group (II)", for use as an inhibitor of glycogen synthase kinase-3, and especially for use in the treatment and/or prophylaxis of conditions associated with a need for the inhibition of GSK-3, such as diabetes and conditions associated with diabetes, chronic neurodegenerative conditions including dementias such as Alzheimer's disease, manic depression, mood disorders such as schizophrenia, neurotraumatic diseases such as acute stroke, hair loss, and cancer; providing that the invention does not encompass a "Compound of Group (II)" for use in the treatment of conditions associated with diabetes mellitus or complications thereof.

The present invention also provides the use of a compound selected from the "Compounds of Group (I)" or the "Compounds of Group (II)" for the manufacture of a medicament for the treatment and/or prophylaxis of conditions associated with a need for the inhibition of GSK-3, such as diabetes and conditions associated with diabetes, chronic neurodegenerative conditions

including dementias such as Alzheimer's disease, manic depression, mood disorders such as schizophrenia, neurotraumatic diseases such as acute stroke, hair loss, and cancer; providing that the invention does not encompass the use of a "Compound of Group (II)" for the manufacture of a medicament for the treatment of conditions associated with diabetes mellitus or complications thereof.

Preferably, a compound selected from the "Compounds of Groups (I)" or the "Compounds of Group (II)" is administered as a pharmaceutically acceptable composition. The compositions of the invention are preferably adapted for oral administration. However, they may be adapted for other modes of administration.

The compositions may be in the form of tablets, capsules, powders, granules, lozenges, suppositories, reconstitutable powders, or liquid preparations, such as oral or sterile parenteral solutions or suspensions.

In order to obtain consistency of administration it is preferred that a composition of the invention is in the form of a unit dose.

Preferably the composition are in unit dosage form. A unit dose will generally contain from 0.1 to 1000 mg of the active compound.

Generally an effectively administered amount of a compound of the invention will depend on the relative efficacy of the compound chosen, the severity of the disorder being treated and the weight of the sufferer. However, active compounds will typically be administered once or more times a day for example 2, 3 or 4 times daily, with typical total daily doses in the range of from 0.1 to 800 mg/kg/day.

Suitable dose forms for oral administration may be tablets and capsules and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinylpyrrolidone; fillers, for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tableting lubricants, for example magnesium stearate; disintegrants, for example starch, polyvinylpyrrolidone, sodium starch glycollate, or microcrystalline cellulose; or pharmaceutically acceptable wetting agents such as sodium lauryl sulphate.

The solid oral compositions may be prepared by conventional methods of blending, filling or tableting. Repeated blending operations may be used to distribute the active agent throughout those compositions employing large quantities of fillers. Such operations are of course conventional in the art. The tablets may be coated according to methods well known in normal pharmaceutical practice, in particular with an enteric coating.

Oral liquid preparations may be in the form of, for example, emulsions, syrups, or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain

conventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, gelatin, hydroxyethylcellulose, carboxymethylcellulose, aluminium stearate gel, hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters such as esters of glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid; and if desired conventional flavouring or colouring agents.

For parenteral administration, fluid unit dosage forms are prepared utilizing the compound and a sterile vehicle, and, depending on the concentration used, can be either suspended or dissolved in the vehicle. In preparing solutions the compound can be dissolved in water for injection and filter sterilized before filling into a suitable vial or ampoule and sealing. Advantageously, adjuvants such as a local anaesthetic, a preservative and buffering agents can be dissolved in the vehicle. To enhance the stability, the composition can be frozen after filling into the vial and the water removed under vacuum. Parenteral suspensions are prepared in substantially the same manner, except that the compound is suspended in the vehicle instead of being dissolved, and sterilization cannot be accomplished by filtration. The compound can be sterilized by exposure to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the compound.

GSK-3 Assay

Types of GSK-3 assay used to test the compounds of the invention include the following:

Type 1: The GSK-3 specific peptide used in this assay was derived from the phosphorylation site of glycogen synthase and its sequence is:

YRRAAVPPSPSLSRHSSPHQ(S)EDEEE. (S) is pre-phosphorylated as is glycogen synthase in vivo and the three consensus sites for GSK-3 specific phosphorylation are underlined. The buffer used to make up the glycogen synthase peptide and [γ - ^{33}P] ATP consisted of MOPS 25mM, EDTA 0.2mM, magnesium acetate 10mM, Tween-20 0.01% and mercaptoethanol 7.5mM at pH 7.00.

The compounds were dissolved in dimethyl sulphoxide (DMSO) to a final concentration of 100mM. Various concentrations were made up in DMSO and mixed with the substrate (GSK-3 peptide) solution (to a final concentration 20uM) described in the above section along with rabbit or human GSK-3 α and GSK-3 β (final concentration 0.5U/ml enzyme). The reactions were initiated with the

addition of [γ - ^{33}P] ATP (500cpm/pmole) spiked into a mixture of ATP (final concentration of 10 μM). After 30 min at room temperature the reaction was terminated by the addition of 10 μl of H_3PO_4 /0.01% Tween-20 (2.5%). A volume (10 μl) of the mixture was spotted onto P-30 phosphocellulose paper (Wallac & Berthold, EG&G Instruments Ltd, Milton Keynes). The paper was washed four times in H_3PO_4 (0.5%), 2 mins for each wash, air dried and the radioactive phosphate incorporated into the synthetic glycogen synthase peptide, which binds to the P-30 phosphocellulose paper, was counted in a Wallac microbeta scintillation counter.

Analysis of Data: Values for IC_{50} for each inhibitor were calculated by fitting a four-parameter logistic curve to the model : $\text{cpm} = \text{lower} + (\text{upper} - \text{lower}) / (1 + (\text{concentration} / \text{IC}_{50})^{\text{slope}})$.

Type 2: This protocol is based on the ability of the kinase to phosphorylate a biotinylated peptide, sequence of which derived from the phosphorylation site of glycogen synthase and its sequence is:

Biot-KYRRAAVPPSPSLSRHSSPHQ(S)EDEEE. (S) is a pre-phosphorylated serine as is glycogen synthase *in vivo* and the three consensus sites for GSK-3 specific phosphorylation are underlined. The phosphorylated biotinylated peptide is then captured onto streptavidin coated SPA beads (Amersham Technology), where the signal from the ^{33}P is amplified via the scintillant contained in the beads.

The kinase was assayed at a concentration of 10 nM final in 25 mM MOPS buffer, pH 7.0 containing 0.01% Tween-20, 7.5 mM 2-mercaptoethanol, 10 mM magnesium acetate, and 10 μM [γ - ^{33}P]-ATP. After 60 minutes incubation at room temperature, the reaction was stopped by addition of 50 mM EDTA solution containing the Streptavidin coated SPA beads to give a final 0.5 mg of beads per assay well in a 384 microtiter plate format.

10 mM stock solutions of the compounds of the invention in 100% DMSO are generated as a first step in the screening process. The second step involves the creation of dose response plates where these compounds are diluted across the plate where the final low and high concentrations are to be 0.008 and 10 μM final in the kinase assay. The third step involves the creation of the assay plates. This is achieved by transferring the compounds from four 96 dose response plates to one 384 assay plate on the Robocon Robolab system. The fourth step is to perform the assay as described and count the resulting plates in the Trilux (Wallac 1450 microbeta liquid scintillation and luminescence counter). The final step is data acquisition and analysis where IC_{50} values are generated for each compound

in duplicate by fitting a four parameter logistic curve to the model : $\text{cpm} = \text{lower} + (\text{upper} - \text{lower}) / (1 + (\text{concentration} / \text{IC}_{50})^{\text{slope}})$ in a batch manner.

The most potent compounds of the present invention show IC₅₀ values in the range of from between 1 to 10 nM.

PKC assay

The PKC peptide used in this assay was a fragment of bovine myelin basic protein (residues 4-14) and this is a specific substrate for protein kinase C. The buffer used to make up the myelin basic protein and [γ -³³P] ATP consisted of Tris 10mM, EGTA 0.9mM, calcium chloride 200uM, magnesium chloride 10mM and a final concentration of 40ug/ml of L-a-phosphatidyl-L-serine and 1ug/ml of 1,3-diolein at pH 7.50.

The test compound was dissolved in dimethyl sulphoxide (DMSO) to a final concentration of 100mM. Various concentrations were made up in DMSO and mixed with the substrate (myelin basic protein) solution (to a final concentration of 0.1mg/ml) described in the above section along with the relevant human recombinant protein kinase C isoform (final concentration of 88mU/ml).

The reactions were initiated with the addition of [γ -³³P] ATP (500cpm/pmole) spiked into a mixture of ATP (final concentration of 10uM). After 20 min at room temperature 15ul of the reaction was spotted onto P-30 phosphocellulose paper (Wallac & Berthold, EG&G Instruments Ltd, Milton Keynes). The paper was washed four times in H₃PO₄ (0.5%), 2 mins for each wash, air dried and the radioactive phosphate incorporated into the myelin basic protein, which binds to the P-30 phosphocellulose paper, was counted in a Wallac microbeta scintillation counter.

No adverse toxicological effects are expected for the compounds of the invention, when administered in accordance with the invention.

The following Examples illustrate the invention, but do not limit it in any way:

Examples

The following examples (shown in Table I with reference to formulae A, B and C) were prepared in accordance with the methods disclosed herein, with particular reference to the publications mentioned above. The activity of the prepared compounds was determined using test methods described herein, including the GSK-3 Assay and PKC assay. The results from the tests are shown in Table I.

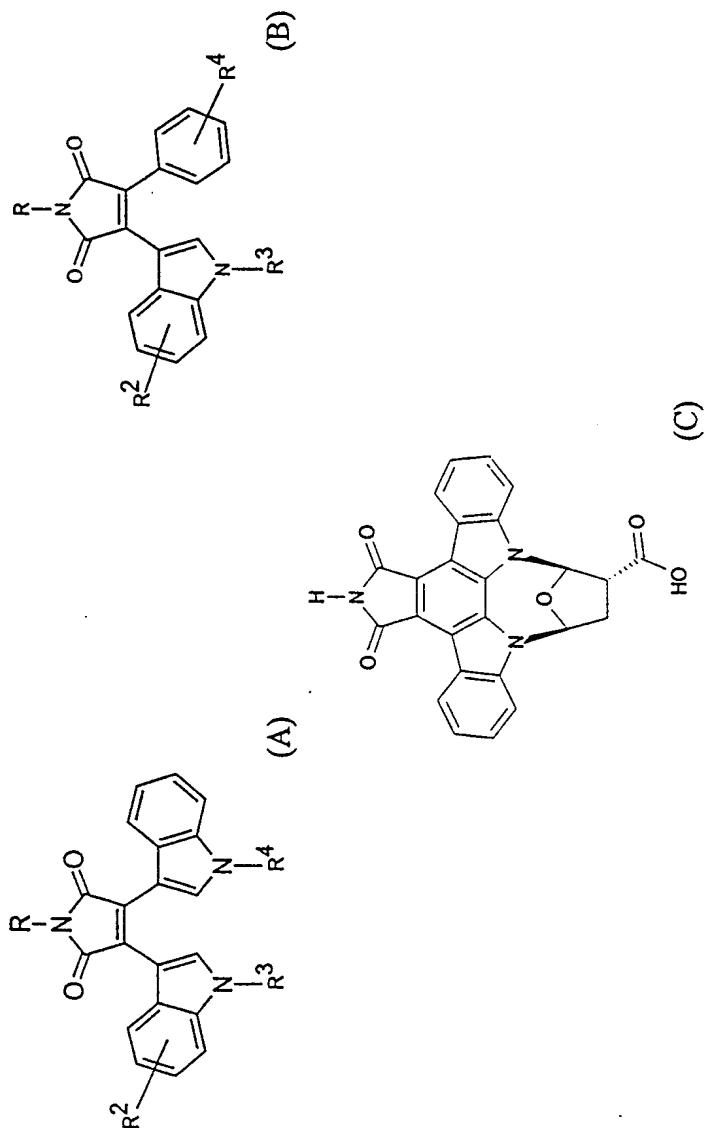


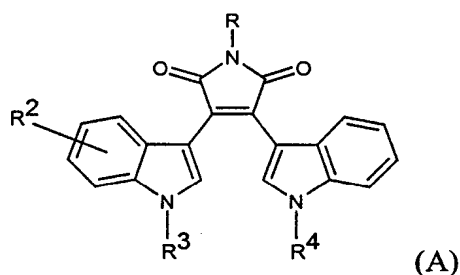
Table I

Formula	Example	R	R2	R3	R4	Rabbit GSK-3 alpha IC ₅₀ (nM)	Human GSK-3 alpha IC ₅₀ (nM)	Human PKC alpha IC ₅₀ (nM)	Human PKC beta2 IC ₅₀ (nM)	Rat brain PKC IC ₅₀ (nM)*
(A)	(A)1	H	H	Me	Me	1.4	60			300+/-60
(A)	(A)2	H	H	Me	n-Pr	9		300	1800	480+/-80
(A)	(A)3	H	H	Me	(CH ₂) ₃ CN	9	22	20	400	
(A)	(A)4	H	H	Me	(CH ₂) ₃ NH ₂	6.5	89	20		75+/-19
(A)	(A)5	H	H	Me	(CH ₂) ₃ COOH	5.8	13	300		
(A)	(A)6	H	H	Me	(CH ₂) ₃ CONH ₂	9.9	34	100	600	
(A)	(A)7	H	5-n-PrO-	Me	(CH ₂) ₃ NH ₂	8.6	93	100	1000	
(A)	(A)8	H	5-Ph	Me	(CH ₂) ₃ OH	8.5	169	90		
(A)	(A)9	H	5-Ph	Me	(CH ₂) ₃ NH ₂	6	48	7.4	400	
(A)	(A)10	H	5-CO ₂ Me	Me	(CH ₂) ₃ OH	5.9	23	4.7	700	
(A)	(A)11	H	5-NO ₂	Me	(CH ₂) ₃ OH	4.7		<16		
(A)	(A)12	H	5-NO ₂	(CH ₂) ₃ OH	Me	5.2	49	350		
(B)	(B)1	H	H	Me	2-Cl	7.5				900+/-400
(B)	(B)2	H	H	Me	2,4-di-Cl	6	87			
(B)	(B)3	H	H	(CH ₂) ₃ OH	2-Cl	3.3	8	400	1400	
(B)	(B)4	H	H	(CH ₂) ₃ NH ₂	2-Cl	5	24	>1000	28700	
(C)	(C)	-	-	-	-	8		1		

* Data for rat brain PKC taken from J Med Chem 1992, 35, 177-184 and 1993, 35, 994-1001

Claims

1. A method for the treatment and/or prophylaxis of conditions associated with a need for the inhibition of GSK-3, such as diabetes and conditions associated with diabetes, chronic neurodegenerative conditions including dementias such as Alzheimer's disease, manic depression, mood disorders such as schizophrenia, neurotraumatic diseases such as acute stroke, hair loss, and cancer, which method comprises the administration of a pharmaceutically effective, non-toxic amount of a compound of formula (I) as respectively defined in EP 470490, WO 93/18766, WO 93/18765, EP 397060, WO 98/11105, WO 98/11103, WO 98/11102, WO 98/04552, WO 98/04551, DE 4243321, DE 4005970, DE 3914764, WO 96/04906, WO 95/07910, DE 4217964, US 5856517, US 5891901, WO 99/42100, EP 328026, EP 384349, EP 540956, DE 4005969, or EP 508792 (the "Publications of Group (I)").
2. A method according to claim 1 wherein the compound of formula (I) is a compound selected from those compounds specifically disclosed as examples in the "Publications of Group (I)".
3. A method according to claim 1 wherein the compound of formula (I) as defined in the "Publications of Group (I)" is of formula (A):



wherein

R is hydrogen;

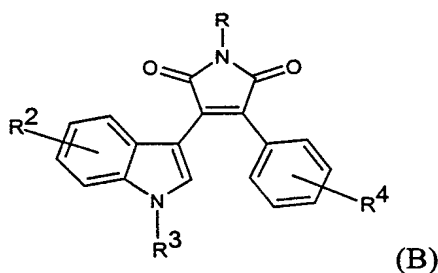
R² is hydrogen, 5-OPrⁿ, 5-Ph, 5-CO₂Me or 5-NO₂;

R³ is Me or (CH₂)₃OH, and;

R⁴ is Me, Prⁿ, -(CH₂)₃X wherein X is selected from CN, NH₂, CO₂H, CONH₂, or OH.

4. A method according to claim 3 wherein the compound of formula (A) is selected from:
- 3,4-bis(1-methyl-3-indolyl)pyrrole-2,5-dione;
- 3-(1-methyl-3-indolyl)-4-(1-propyl-3-indolyl)pyrrole-2,5-dione;
- 3-(1-methyl-3-indolyl)-4-(1-[3-cyanopropyl]-3-indolyl)pyrrole-2,5-dione;
- 3-(1-methyl-3-indolyl)-4-(1-[3-aminopropyl]-3-indolyl)pyrrole-2,5-dione;
- 3-(1-methyl-3-indolyl)-4-(1-[3-carboxypropyl]-3-indolyl)pyrrole-2,5-dione;
- 3-(1-methyl-3-indolyl)-4-(1-[3-carbamoylpropyl]-3-indolyl)pyrrole-2,5-dione;
- 3-(1-methyl-5-propyloxy-3-indolyl)-4-(1-[3-aminopropyl]-3-indolyl)pyrrole-2,5-dione;
- 3-(1-methyl-5-phenyl-3-indolyl)-4-(1-[3-hydroxypropyl]-3-indolyl)pyrrole-2,5-dione;
- 3-(1-methyl-5-phenyl-3-indolyl)-4-(1-[3-aminopropyl]-3-indolyl)pyrrole-2,5-dione;
- 3-(1-methyl-5-methoxycarbonyl-3-indolyl)-4-(1-[3-hydroxypropyl]-3-indolyl)pyrrole-2,5-dione;
- 3-(1-methyl-5-nitro-3-indolyl)-4-(1-[3-hydroxypropyl]-3-indolyl)pyrrole-2,5-dione, and;
- 3-(1-[3-hydroxypropyl]-5-nitro-3-indolyl)-4-(1-methyl-3-indolyl)pyrrole-2,5-dione;
- or a pharmaceutically acceptable derivative thereof.

5. A method according to claim 1 wherein the compound of formula (I) as defined in the "Publications of Group (I)" is of formula (B):



wherein

R is hydrogen;

R² is hydrogen;

R³ is Me or a group -(CH₂)₃Y wherein Y is NH₂ or OH, and;

R⁴ is 2-Cl or 2,4-di-Cl.

6. A method according to claim 5 wherein the compound of formula (B) is selected from:

3-(1-methyl-3-indolyl)-4-(2-chlorophenyl)pyrrole-2,5-dione;
3-(1-methyl-3-indolyl)-4-(2,4-dichlorophenyl)pyrrole-2,5-dione;
3-(1-[3-hydroxypropyl]-3-indolyl)-4-(2-chlorophenyl)pyrrole-2,5-dione, and;
3-(1-[3-aminopropyl]-3-indolyl)-4-(2-chlorophenyl)pyrrole-2,5-dione;
or a pharmaceutically acceptable derivative thereof.

7. A method according to claim 1 wherein the compound of formula (I) as defined in the "Publications of Group (I)" is 9,10,11,12-tetrahydro-10-carboxy-9,12,-epoxy-1H-diindolo[1,2,3-fg:3',2',1'-kl]pyrrolo[3,4-i]benzodiazocine-1,3(2H)-dione or a pharmaceutically acceptable derivative thereof.

8. A method for the treatment and/or prophylaxis of conditions associated with a need for the inhibition of GSK-3, such as diabetes and conditions associated with diabetes, chronic neurodegenerative conditions including dementias such as Alzheimer's disease, manic depression, mood disorders such as schizophrenia, neurotraumatic diseases such as acute stroke, hair loss, and cancer which method comprises the administration of a pharmaceutically effective, non-toxic amount of a compound of formula (I) as defined in WO 95/17182, WO 95/35294, EP 624586, EP 657458, EP 776899, EP 805158, US 5491242, US 5541347, US 5545636, US 5552396, US 5624949, US 5710145, US 5721272, WO 97/18809, or WO 98/07693 (the "Publications of Group (II)"), but providing that the method does not encompass the treatment of conditions associated with diabetes mellitus or complications thereof.

9. A method according to claim 8 wherein the compound of formula (I) is a compound selected from those compounds specifically disclosed as examples in the "Publications of Group (II)".

10. A compound of formula (I) as defined in any one of claims 1-9, for use as an inhibitor of glycogen synthase kinase-3, and especially for use in the treatment and/or prophylaxis of conditions associated with a need for the inhibition of GSK-3, such as diabetes and conditions associated with diabetes, chronic neurodegenerative conditions including dementias such as Alzheimer's disease, manic depression, mood disorders such as schizophrenia, neurotraumatic diseases such as acute stroke, hair loss, and cancer; providing that the compounds of formula (I) as defined in claims 8-9 are excluded from use in the treatment of conditions associated with diabetes mellitus or complications thereof.

11. Use of a compound of formula (I) as defined in any one of claims 1-9 for the manufacture of a medicament for the treatment and/or prophylaxis of conditions associated with a need for the inhibition of GSK-3, such as diabetes and conditions associated with diabetes, chronic neurodegenerative conditions including dementias such as Alzheimer's disease, manic depression, mood disorders such as schizophrenia, neurotraumatic diseases such as acute stroke, hair loss, and cancer; providing that the compounds of formula (I) as defined in claims 8-9 are excluded from the manufacture of a medicament for the treatment of conditions associated with diabetes mellitus or complications thereof.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 99/04374

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 A61K31/40 A61P3/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 328 026 A (HOFFMANN LA ROCHE) 16 August 1989 (1989-08-16) cited in the application page 9, line 11-14 page 38, line 7; claim 9 ---	1-7, 10, 11
X	EP 0 470 490 A (HOFFMANN LA ROCHE) 12 February 1992 (1992-02-12) cited in the application page 3, line 38-42 ---	1-7, 10, 11
X	US 5 545 636 A (SCHOTTEN THEO ET AL) 13 August 1996 (1996-08-13) cited in the application column 1 -column 3 --- -/--	8-11

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

9 March 2000

Date of mailing of the international search report

24.03.00

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Brunnauer, H

INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/GB 99/04374

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 710 145 A (ENGEL GARY LOWELL ET AL) 20 January 1998 (1998-01-20) cited in the application column 1 -column 2 ----	1-7,10, 11
X	US 5 541 347 A (WINNEROSKI II LEONARD L ET AL) 30 July 1996 (1996-07-30) cited in the application column 1 ----	8-11
A	WO 97 41854 A (UNIV PENNSYLVANIA ;PRESIDENTS AND FELLOWS OF HARV (US)) 13 November 1997 (1997-11-13) cited in the application page 9, line 4-11 page 47; claim 1 ----	1-11
A	WO 98 16528 A (CHIRON CORP ;UNIV CALIFORNIA (US)) 23 April 1998 (1998-04-23) page 21; claims 26,27 -----	1-11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 99/04374

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-2 and 8-11 (in part)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-2 and 8-11 (in part)

Present claims 1-2 and 8-11 relate to an extremely large number of possible compounds defined by references to patent numbers. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds according to claims 3-7.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 99/04374

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 99/04374

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